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DIVERSITIES AT ENDOMIXIS
IN PARAMECIUM AURELIA

By

LUCILE JANE CALDWELL

A DISSERTATION

SUBMITTED TO THE BOARD OF UNIVERSITY STUDIES OF THE
JOHNS HOPKINS UNIVERSITY IN CONFORMITY WITH
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

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ONE FIGURE

INTRODUCTORY

Erdmann ('20) has given direct evidence of the production of inherited diversities in size at endomixis in *Paramecium aurelia*. Other investigators have given indirect evidence that endomixis may result in inherited diversities, or have expressed the opinion that this is probable. The present paper is a detailed investigation of the production at endomixis of physiological and morphological diversities in the stock of *Paramecium aurelia* that has been under investigation for several years in the zoölogical laboratory of The Johns Hopkins University.

In the work of Erdmann ('20), a clearly marked difference in size arose in a clone of *Paramecium aurelia* at endomixis. The two products of the first fission of a single individual after the climax of endomixis gave rise to two sets of lines, A and B. The individuals of these two lines differed in mean length, those of A showing a length of 38.7 units, as compared with 42.7 units in B. These differences persisted through five later comparisons extending over a period of nearly 100 generations. In certain other cases Erdmann found very slight differences in size in different lines arising at endomixis; these also she believes due to the nuclear changes in that process.

¹From the Zoölogical Laboratory of The Johns Hopkins University. This investigation was begun in collaboration with Prof. H. S. Jennings, but was completed during his absence in Japan. The author is indebted to Dr. T. M. Sonneborn for suggestions during the work, and to Doctor Sonneborn and Professor Jennings for aid in preparing the paper.

Jollos ('21) reports the disappearance at endomixis of 'Dauermodifikationen' that had been induced by environmental action combined with selection. Characteristics that had been inherited for many generations thus often disappeared after the animals had gone through endomixis.

Some indirect evidence on the relation of change in hereditary characteristics to endomixis has been obtained by Parker ('27). Working on the results of selection of diversities, Parker found that he could obtain inherited diversities within clones of *Paramecium aurelia*, in which endomixis occurs, but not in *Paramecium calkinsi*, in which endomixis does not occur. He is inclined to believe, therefore, that the constitutional diversities arose at endomixis. Slight inherited differences were found to arise also in clones of *Stylonychia pustulata* (as had before been discovered by Middleton, '15), and in this animal no endomictic process has been observed in vegetative life. Parker is inclined to hold, however, that some process of nuclear reorganization may yet be found during the vegetative life of *Stylonychia*.

Erdmann ('22) is disposed to attribute very great significance to the inherited variations resulting from endomixis and related processes, holding that they supply the basis for evolutionary changes in Protozoa. A résumé and discussion of the facts and theories touching this matter is given by Jennings ('29, pp. 200-203 and pp. 304 ff.).

The stock of *Paramecium aurelia* cultivated in the Johns Hopkins Zoölogical Laboratory since July 29, 1929 (at which time it was derived from fission of a single individual), has shown many phenomena suggesting the possibility of the origin of heritable variations at endomixis. In addition to the great number of heritable diversities arising at conjugation (as described by Jennings, Raffel, Lynch, and Sonneborn, '32), less numerous heritable diversities have been found to arise during vegetative reproduction (compare the paper just cited, pp. 403-405, and Raffel, '32). There have been indications that some of these take origin during endomixis.

A systematic study of this matter was therefore undertaken. Special attention was devoted to changes in fission rate after endomixis; but other physiological and morphological characteristics were likewise taken into consideration. The results of this study are herewith presented.

MATERIAL AND METHODS

As remarked above, the observations set forth in the present paper were all made upon the stock of *Paramecium aurelia* derived from the fission of a single individual on July 29, 1929. This stock, originally a single clone, has passed through many conjugations, giving rise to many clones with diverse hereditary characteristics, as described in the various recent papers of Raffel, Jennings, Sonneborn, and Lynch (see the literature cited). In the present study, single clones of this stock were employed, each derived by vegetative fission from a single individual. No conjugation occurred during the course of the observations.

Throughout the work, daily isolation cultures were employed. Each individual was kept in a separate drop of culture fluid on a double depression slide in a moist chamber. At the same time each day (in so far as was possible), after the number of fissions occurring during the past 24 hours had been recorded, one individual from each drop was transferred to a drop of fresh culture fluid on a clean slide. The culture fluid used was an oatmeal infusion prepared according to the formula of Jennings et al. ('32): 30 flakes of Quaker rolled oats were boiled for 3 minutes in 100 cc. of spring water and filtered. This filtrate was ready for use 24 hours later, by which time a bacterial flora had accumulated; then it was inoculated with the alga *Stichococcus bacillaris*, grown on agar slants. (For details of culture of this alga, see Raffel, '30.) This inoculated fluid was prepared daily. The earlier experiments were kept at room temperatures, the later ones in a controlled temperature box, with temperatures at 23° to 25°C.

The most prominent feature of individuals undergoing endomixis lies in the presence of the fragmented macronuclei. This was used throughout the present work as the test for endomixis; individuals containing macronuclear fragments were considered to be undergoing the endomictic process. Examination was made by killing and fixing the individuals and staining them with 36 per cent aceto-carmin.

A large number of lines were kept under cultivation, making it impossible to thus examine cytologically a representative of every line each day, as was attempted at the beginning. On the basis of the experience gained in these daily examinations, it became possible to devise rules which would make the examinations unnecessary in periods in which there was no possibility of endomixis. Those rules, adhered to in the later work, were as follows:

- 1) Unless suspected of endomixis on other grounds, lines which had divided three times during the last 24 hours were not cytologically examined;
- 2) lines which underwent the last previous endomixis 17 or more days ago were examined daily;
- 3) lines in which the cytoplasm appeared in any way abnormal were examined;
- 4) lines ordinarily dividing two to three times a day, which had divided only once during the past 24 hours were invariably examined;
- 5) lines in which the usual size of 160 by 70 was increased to about 200 by 80 were also invariably examined.

By rigid adherence to these rules, much useless labor was avoided and it was possible to experiment with larger numbers of lines without seriously impairing the probability of discovering exactly when endomixis was occurring.

In all lines, whenever examination was made of two or more sister individuals, one was retained to perpetuate the line, while the others were subjected to cytological study.

Studies were made on two different clones (both derived, as before seen, from the same original stock produced by a single individual). Clone I was derived from a single individual, obtained February 16, 1931, from Sonneborn's clone E40a. This was expanded as quickly as possible to forty-

eight lines which were cultivated until February 27th, when all were discarded except one, designated A41. This was at once expanded to ninety-six lines, which were kept in cultivation until May 25, 1931. During this time any line that died out was replaced from a flourishing sister line.

Clone II was derived from a single individual, taken October 22, 1931, from Sonneborn's clone BN9b 12a. This was at once expanded to eighteen lines, and these were kept in continuous isolation culture until January 25, 1932. During this time five successive endomictic periods occurred. In contrast to the procedure with clone I, after each endomixis in clone II some lines were expanded and, usually, some discarded. After the first endomixis (October 28th to November 3rd), only a few lines were expanded and these not extensively; no lines were discarded. About 10 days after the second endomixis (November 17th to 23rd) some lines were more extensively expanded, bringing the total to 100 lines. After the third endomixis (December 5th to 15th), each line was expanded according to the following rule. The day after fragmentation was first discovered the line was expanded to as many lines as there were individuals present. For example, if the one individual kept on the first day of fragmentation had multiplied to four by the next day, the one line was expanded to four lines. In some cases, if there were any indication of the appearance of an altered characteristic, the process of expansion was continued for several days after endomixis. Later, in many cases, if there were no marked indications that lines had been altered by endomixis, they were discarded. In the later endomixes, similar expansions and rejections were made. A chart of the different divisions of clone II, with their designations, is given in table 1.

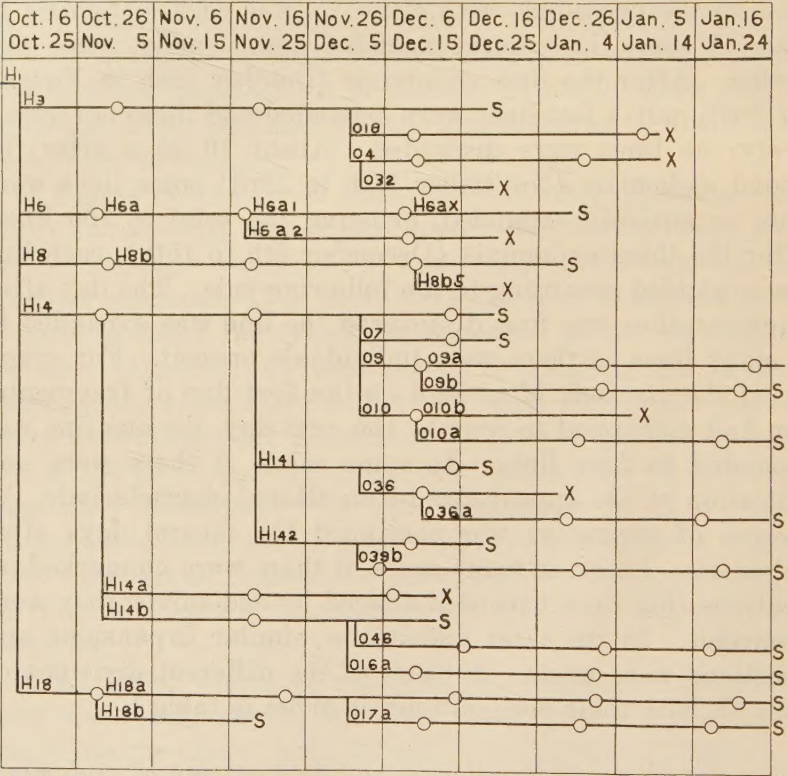
1. Constitutional diversities in viability arising at endomixis

As is well known, there is often a great increase in mortality during the process of endomixis. In some of the clones of *Paramecium aurelia* many of the lines under culture die out at this time (Jennings, Raffel, Lynch, and Sonneborn, '32,

pp. 401-403). As will be shown later, a part of this mortality is due to the existence of clones that differ in viability, some being so weak as to be unable to go through the complex process of endomixis. We shall deal first with the general situation as to mortality at endomixis.

TABLE 1

Chart or diagram showing the origin and history of the various lines descended from clone II, with the periods at which endomixis occurred (indicated by a small black circle). An X shows the time at which the line died. An S signifies that the line was not cultivated beyond the date indicated.



The processes of nuclear change in endomixis may last, as is well known, through about ten generations. For comparing the mortality during endomixis with that occurring at other times, we may then compare the death rate for the ten gen-

erations from the time endomixis begins with the ten generations in the middle of the intermictic period, when endomixis is not in progress.

The results show a remarkable contrast in mortality between the two clones, though in both the mortality is greatly increased during endomixis.

In clone I the mortality was studied in 283 lines. Among these, death occurred 160 times during the 10 days following the beginning of endomixis, and only 17 times during 10 days in the middle of the intermictic period. The mortality during the endomictic period was thus 56.54 per cent, while in the intermictic period it was but 6.01 per cent. The death rate in clone I is thus during endomixis 9.40 times greater than the intermictic period.

In clone II, among 373 lines observed, 101 died during the 10 days following the beginning of endomixis—a death rate of 27.08 per cent. In the 10 days in the midst of the intermictic period, twenty-nine lines died—a death rate of 10.66 per cent. The death rate in clone II during endomixis is thus 2.85 times the death rate in the intermictic period.

As the above figures show, the death rate during endomixis is, in clone I, 2.09 times as great as the corresponding death rate in clone II. This greater death rate at endomixis in some clones than in others was noted in general terms by Jennings, Raffel, Lynch, and Sonneborn ('32, p. 402).

A detailed examination of the mortality data shows that the endomictic process produces certain lines that are non-viable, while in the same clone other lines are produced that are viable. In other words, endomixis has different effects on the constitution of the different lines that go through it, causing in some a change that results in the dying out of the lines. Evidence on this will now be presented.

The completion of the process of endomixis may require as much as nine or ten successive generations. Thus while the process is occurring, a single individual may produce many lines of descent (up to 1024 in ten generations). In some cases all the lines of descent from a particular individual

that undergoes endomixis die out within a few generations, showing that endomixis in this case produced inviable lines. Under the same conditions the descendants of other individuals that have undergone endomixis survive and propagate.

Examples of non-viable lines produced by endomixis are seen in lines 032, H14a, 018, and 04, all belonging to clone II (table 1). At endomixis, the line 032 was expanded to six lines, of which all died in 9 or less days after endomixis was first observed; not more than seven generations had passed in any line. Conditions in H14a were similar; here seven lines were set up and all died within 8 days after not more than five generations following the first evidence of endomixis. The full record of divisions and death in line H14a is given below (X signifying death of the line):

Dec.	4	5	6	7	8	9	10	11	12	13	14	15	16	
	2	1	2	0	1	1	2	1	X					
									0	0	0	X		
									0	0	0	0	X	
									0	0	0	0	X	
									0	2	1	0	0	X
									0	0	0	0	0	X
									0	0	0	0	0	X

The conditions in lines 018 and 04 were somewhat different and are of particular interest. These two lines were branches taken from the line H3 on December 4th. After the endomixis of December 13th to 15th, the line 018 was expanded to seventeen lines, 04 to two lines. After the next endomixis, which began between December 28th and January 7th, all lines of both 04 and 018 died out. At this time, the lines of 04 were expanded to fourteen lines, of which none lived longer than nine generations or 9 days after the first observation of endomixis. Among the descendants of 018, seven lines living before this last endomixis were not expanded; all of these died within 5 days (two generations) after the beginning of

endomixis. The other ten lines were expanded to twenty-five lines after the onset of endomixis, of which all died within six generations (11 days).

Thus in these cases all the descendants of particular lines died following endomixis in the line. In one of the cases, a great number of lines of descent from a common ancestor showed the same effect of endomixis in producing non-viability. Clearly, at endomixis, in these cases, a profound alteration in the genetic constitution has occurred, resulting in the extinction of all the lines of descent from these particular individuals.

In other cases a single line is split at endomixis into viable and non-viable sub-lines. This is illustrated in line H6a of clone II. This line began to undergo endomixis on December 9th, and was thereupon expanded into twelve lines. Of these six died within eight generations (7 days), while the other six continued to multiply and remained normal in every way. The record of daily fissions for this line H6a and its branches follows (the letter X signifying death of the line):

Dec	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	1	2	2	1	2	2	1	X 2 { 3 2 3 2 3 2 2 2 etc. 3 1 3 3 3 2 2 2 etc. 2 3 1 3 2 3 2 3 1 etc. } 1 { 0 3 { 2 1 0 X 2 { 1 X 0 X } } 1 { 1 1 3 2 2 3 etc. 1 1 3 2 2 3 etc. } 1 { 1 2 2 2 2 2 etc. 1 { 0 0 0 X 0 0 0 X } }								

Another type of effect of endomixis on mortality is illustrated by line H6a2 and its branches. In this line, endomixis

began on December 8th and quickly thereafter the line was expanded into seventeen lines. Of these, thirteen manifested reduced vigor and eventually died, but not at once. None died before seven generations had elapsed after endomixis was first observed. And none passed through more than thirteen generations after this observation. Thus some died during the process of endomixis, some died soon afterward. Four other lines of H6a2 lived and continued to propagate vigorously after endomixis.

For comparison with the above may be cited the history of line 046. This underwent endomixis on December 16th and was soon expanded to four lines. All of these lines went through endomixis and the following intermictic period in full vigor without the death of any lines.

In sum, therefore, the following facts are shown as to the mortality resulting from endomixis. 1) Mortality is in some clones many times greater during the ten generations following the beginning of endomixis than at other periods. 2) Some lines are made by endomixis constitutionally non-viable; they divide a number of times during endomixis, but all the resulting lines die within a few generations. 3) Some lines are differentiated at endomixis into viable and non-viable descendant lines. 4) Some of the lines thus produced live with reduced vigor for some generations after completion of the process of endomixis, but die soon. 5) Some clones go through endomixis with undiminished vigor, and without death in any of their branches.

2. Hereditary diversities in fission rate arising at endomixis

An extensive series of observations was made in clones I and II on the appearance of diversities of fission rate in consequence of endomixis. These observations gave unexpectedly complete evidence of the not infrequent occurrence of changes in fission rate after endomixis, and of the production in this way, within a single clone, of lines with diverse fission rates.

We shall first deal statistically with the variations in fission rate shown by a clone after each of a series of endomictic periods. Later we shall take up the changes shown by particular lines, and the types of change occurring at endomixis, with examples.

A. Comparison of the variability in fission rate after each of a series of successive endomictic periods. Does the variability in fission rate show any increase or other definable change, in consequence of endomixis? Four successive endomixes were observed in clone I, five in clone II. The variability in fission rate was determined after each endomixis: the results are presented in table 2. In this table are given the means, standard deviations, and coefficients of variation of the daily fission rates, determined for a 5-day period lying midway between two endomictic periods. For interpreting these, consideration of the method of procedure in each case is essential.

In clone I, one individual, A41, underwent endomixis on February 20th, gave rise to ninety-six lines, fifty-nine of which are included in the group examined in the first period listed in table 2. Of these, twenty-six lived through the later endomixis of March 9th to 18th, and they and their descendants, making fifty-six lines in all, are included in the group examined in the second period. Fifty-three of these lines died out or were discarded; three were selected and kept. Of these three, one line showing a lowered fission rate was expanded to fifteen lines; and two lines showing a raised fission rate were expanded, one also to fifteen lines, the other to six lines. These underwent endomixis April 3rd to 12th, and make up the group examined in the third period. Twelve of these and their descendants, making twenty lines in all, underwent a third endomixis, and are included in the group examined in the fourth period. Seven of these underwent a fourth endomixis, and they and their descendants, fourteen lines in all, are included in the group examined in the fifth period. These last fourteen lines were descended from only two of the original fifty-nine lines examined in the first period.

In clone II, ten lines, all descended from one individual which presumably underwent endomixis prior to October 16th, are included in the group examined in the first period (October 18th to 22nd). Nine of these underwent the first endo-

TABLE 2

Comparison of the variabilities in fission rate shown by two clones after each of a series of successive endomixes. For each 5-day intermictic period are given the limiting dates; the number of lines considered, their mean daily fission rate, and the maximum and minimum rates observed; the standard deviation and its relative value, using the value for the first period as unity; and the coefficient of variation and its relative value, using the coefficient of the first period as unity. The first period in each case precedes by 4 days or less the first endomictic period observed; the other periods are midway between two endomixes.

PERIOD	DATES, 1931-1932	NUMBER OF LINES	MEAN FISSION RATE	MAXIMUM AND MINIMUM FISSION RATES	STANDARD DEVIATION		COEFFICIENT OF VARIATION		
					Absolute value	Relative value	Absolute value	Relative value	
Clone I									
1	Mar. 5-9	59	2.64±0.02	3.0 2.0	0.20±0.01	1	7.61±4.75	1	
2	Mar. 20-24	56	2.65±0.03	3.2 1.0	0.37±0.02	1.8	23.77±1.60	3.1	
3	Apr. 21-25	37	1.61±0.06	2.4 0.6	0.52±0.04	2.5	30.55±2.61	4.0	
4	May 6-10	20	1.78±0.10	2.8 0.8	0.68±0.07	3.4	38.91±4.97	5.1	
5	May 19-23	14	1.13±0.12	2.8 0.4	0.67±0.08	3.3	59.08±9.81	7.8	
Clone II									
1	Oct. 18-22	10	1.58±0.04	1.8 1.4	0.17±0.03	1	10.51±1.60	1	
2	Nov. 12-16	31	1.54±0.02	2.0 1.2	0.18±0.02	1.1	11.97±1.04	1.1	
3	Nov. 24-28	18	2.09±0.04	2.4 1.8	0.27±0.03	1.5	12.70±1.44	1.2	
4	Dec. 18-22	54	1.90±0.04	2.4 0.6	0.44±0.03	2.6	23.03±1.57	2.1	
5	Jan. 6-10	37	1.75±0.06	2.6 0.4	0.58±0.05	3.5	33.30±2.89	3.2	
6	Jan. 21-25	54	1.35±0.05	2.4 0.4	0.56±0.04	3.4	41.74±3.40	3.9	

mixis observed in this clone and they and their descendants, thirty-one lines in all, are included in the group examined in the second period (November 12th to 16th). Thirteen of these, descended from eight of the original ten lines, underwent the second observed endomixis, and they and their descendants, eighteen lines in all, are included in the group examined in the third period (November 24th to 28th). These were further expanded to sixty-five lines, eighteen of which, still representing eight of the original ten, underwent the third observed endomixis, and are included with their descendants in the group examined in the fourth period. These were further expanded to 116 lines. In five groups of these lines, representing one of the original ten individuals of the clone, and in one other group of lines, representing another one of the original ten, the fourth endomixis was observed, and these groups include the lines examined in the fifth period. The fifth and also the last endomixis was observed in three of these groups, descended from one of the original ten, and in one other group, descended from another of the original ten, and these were examined in the sixth and last period.

It is obvious that selection was practiced in choosing the lines on which the constants of variability are based. The purpose was to determine whether there are statistical indications that lines diverse in fission rate are at endomixis produced from a uniform clone. To determine this, after endomixis lines that gave indications of diversity were further tested, including those lines which appeared higher, and those that appeared lower in fission rate. In clone I no selection was made until after the second examination period (March 20th to 24th). But before the third examination period, many of these lines were discarded, only three being selected for further examination. Of these three, one showed a lowered fission rate; two showed a raised fission rate. Before the fifth examination period, one of the two lines showing the raised fission rate had died; so that the lines used in the fifth period were descended from two lines, one selected for high fission rate, one selected for low fission rate. Clone II

was similarly treated, expansion of some lines, and discarding of others preceding the fourth examination period and continuing through the rest of the experiment.

For both clones, fission rates were calculated for periods of 5 days, lying as nearly as possible midway between two succeeding endomixes. In clone I, the first period (March 5th to 9th) immediately preceded the first observed endomixis (March 9th to 18th). In clone II, the first period preceded the first observed endomixis by 4 days. All other periods in both clones lay midway between two known endomixes, so that the lines used in the calculations were neither beginning nor completing endomixis. The results are, therefore, unaffected by the temporary lowering of fission rate which frequently appears at endomixis.

As table 2 shows, there was a consistent rise in the values of the measures of variation in both clones, as they passed through successive periods of endomixis. The standard deviation is in both clones 3.3 times as great after the four (or five) periods of endomixis, as compared with what it was at the beginning. The coefficient of variation shows a still greater increase, owing to the fall in the mean fission rate, resulting from the more frequent production of lines with lowered fission rate than of those with higher fission rate. The increase in the standard deviation is therefore the better measure of the increased variation.

Thus the variability in fission rate increases greatly as the clones pass through successive endomictic periods. Little significance is of course to be attached to the absolute values of the coefficients, since the higher and lower lines were selected for continued propagation within each clone. But higher and lower lines could be selected only because they appeared after endomixis. If there had been no selection, there must therefore still have been an increase in variation, although the coefficients might have been less in amount. It is clear that passage through a series of endomictic periods increases the variability of the fission rate. The precise nature of the changes is to be investigated in the following sections.

B. Comparison before and after endomixis of lines whose fission rates were similar before endomixis, but diverse after endomixis. It was found that after endomixis certain of the lines had changed in fission rate, so that lines which had the same fission rate before endomixis differed in fission rate after endomixis. Cases of this sort are shown in tables 3 and 4.

TABLE 3

Comparison of fission rates of lines of clone I before and after endomixis of March 10 to 16, 1931

LINES	MARCH 5-9	ENDOMIXIS March 10-16	MARCH 17-21	MARCH 22-26	MARCH 27-31	MEAN OF MEANS
C 24	2.40		2.53	3.00	3.40	2.98
C 74	2.60		1.80	2.20	2.20	2.07
C 75	2.80		0.87	0.47	...	0.67

In table 3 are compared the fission rates of certain lines of clone I, before and after the endomixis of March 10th to 16th. Before fission the three lines showed practically the same fission rates, though for the 5-day period there were small differences, the daily rates being 2.40, 2.60, and 2.80. After endomixis the three lines differed to a marked degree in their fission rates, and these differences persisted throughout the three 5-day periods. One of the lines (C24) has after endomixis a daily fission rate of 2.50 to 3.40; another (C75) a daily rate of but 0.47 to 0.87, while the third (C74) is intermediate.

Table 4 gives similar data for four lines of clone II, before and after the endomixis of December 9th to 15th; in this

TABLE 4

Comparison of fission rates of lines of clone II before and after endomixis of December 9 to 15, 1931

LINES	NOV. 22-26	NOV. 27- DEC. 1	DEC. 4-8	MEAN OF MEANS	ENDO- MIXIS	DEC. 17-21	DEC. 22-26	DEC. 27-31	MEAN OF MEANS
H6ax	2.20	1.60	1.60	1.80		2.60	2.20	2.40	2.40
07	2.00	2.00	1.80	1.93		...	1.80	2.00	1.90
010b	2.00	2.00	1.80	1.93		1.40	1.40	1.40	1.40
09a	2.00	2.00	1.80	1.93		1.20	0.60	1.00	0.93

case the fission rates for the four lines are given for three 5-day periods before endomixis and three after endomixis. Before endomixis the four lines had practically the same fission rates, the average of one, however (H6ax), falling a little below that of the others. After endomixis the four lines differed in their fission rates to a marked degree. The mean fission rate in the line H6ax is now uniformly more than twice that in the line 09a, while the lines 07 and 010b fall between these two. If the mean rate for 09a is taken as unity, then the rates for the four are (reading from below upward in table 4), respectively, 1, 1.5, 2.0, and 2.6. Thus the four lines practically identical in their fission rates were differentiated by endomixis into four lines with very different rates.

It will be understood, of course, that in many cases endomixis leaves the lines with fission rate practically unchanged. The cases shown in tables 3 and 4 demonstrate, however, that in some cases endomixis results in changes in the fission rate.

C. Types of hereditary change in fission rate arising at endomixis (tables 5 to 8). The hereditary changes in fission rate arising at endomixis differ, in different cases, in direction, magnitude, and duration. In this investigation, the only changes studied were those that were great enough to show clearly without resort to refined statistics. In view of the fact that many such changes were discovered, it seems probable that many additional smaller changes could have been demonstrated if large enough numbers of the altered lines had been studied to permit of statistical treatment. Among the large changes studied, some were to higher fission rates than before endomixis, but most were to lower rates. This is possibly due to the fact that both clones investigated had, to begin with, fission rates near the maximum found in *P. aurelia* under these conditions of cultivation. Among the many changes to lower fission rates that occurred, some lasted only a short time, ending with the death of the lines during or shortly after endomixis; others lasted though an entire inter-mictic period, terminating with the death of the lines at the next endomixis or else their return to the former fission rate;

and a few lasted through at least two intermictic periods, or even farther. Examples of all these types of changes will be given.

Since the endomictic processes of any one period may continue through nine or ten vegetative generations, at the end of the process there may have arisen from a single individual in which endomixis began 500 to 1000 lines of descent. Only a small number of these can be kept. Thus the fact that in some cases lines emerging from endomixis differ from the parent line that entered endomixis of course does not show that in all the lines this difference would occur. Indeed, in many cases we know that after endomixis some lines differ from the parent line, while others remain like it. In our examples of differences arising at endomixis, in tables 5 to 8, some are cases in which two or more lines descended from the same parent line differ; while others are cases in which the descendants of two similar parent lines that have both undergone endomixis are found after endomixis to differ.

(a) Changes to lower fission rate, lasting only a short time and terminated by the death of all the changed lines. Two examples of this type of change will be given. The first is illustrated by the history of line H8b of clone II, given in table 5. For the four 5-day periods prior to endomixis on December 9th, the mean fission rates of lines H8b were 2.0, 2.4, 2.4, and 1.8 fissions per day. During the first five days of endomixis the mean fission rate was 2.0 fissions per day. In this period the line was expanded to six lines (nos. 1 to 6); subsequently, one of these lines (6) showed a marked decrease in fission rate and was expanded to eleven lines (nos. 7 to 16), making a total of sixteen. Lines 1 to 5 continued to manifest the characteristic high fission rate until discarded. Lines 3, 4, and 5 were discarded 8 days after the beginning of endomixis, while lines 1 and 2 were kept till 17 and 18 days, respectively, after the beginning of endomixis. The fission rate of line 1 for this whole period was 2.39 fissions per day, and this was typical of all the five. The low fission rate of line 6 was first manifested on the fifth day after endomixis

began; this was ten generations after the beginning of endomixis and so places the change at the end of the endomictic process. The change persisted till the death of all lines derived from line 6. These eleven lines died in from 8 to 15 days (mean, 9.9 days) after the change. During this time the lines passed through four to seven generations (mean 5.45). Their mean fission rate for this period was thus 0.55

TABLE 5
Fission rate history of line H8b

DECEMBER, 1931																												LINES
9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28									
2	{	1	2	3	2	2	3	3	1	2	2	2	3	3	3	2	2	3	1	S	1							
		2	3	2	2	2	1	2	3	0	2	3	2	3	2	3	2	S		2								
{	1	2	2	2	3	2	2	3	S											3								
		2	2	3	2	3	2	3	S											4								
{	2	3	2	3	2	3	1	S												5								
		3	2	1	1	1	1	1	1	1	0	0	0	0	1	0	0	0	X	6								
{	1	1	0	0	0	0	0	0	0	1	0	0	0	X						7								
		1	0	0	0	0	0	0	X											8								
{	1	0	0	0	0	0	X													9								
		0	0	0	X															10								
{	1	0	0	0	X															11								
		0	0	0	X															12								
{	1	1	1	0	0	X														13								
		1	0	1	0	X														14								
{	1	0	1	0	0	X														15								
		0	0	0	X															16								

X signifies that the line died out at the date so marked, while S signifies that the line was discarded while still flourishing. For the four 5-day periods prior to endomixis on December 9th, the daily fission rates were 2.0, 2.4, 2.4, and 1.8.

fissions per day, as compared with 2.39 fissions for the unaltered sister lines. During the last three periods shown in table 5 (December 14th to 27th) the fission rates were for the unaltered lines 2.3, 2.2, and 2.4, while for the same 5-day periods the means for the altered lines were 1.0, 0.2, and 0.2, respectively. Thus the lowered fission rate induced by endomixis was continued throughout the periods, and was terminated only at the death of the altered lines.

TABLE 6
Fission rate history of line 036

DECEMBER, 1931																															LINES
9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31									
2	1	3	1	{	1	2	3	2	2	3	1	2	2	3	2	3	2	2	3	2	1	1	1	1							
					2	3	1	2	3	S																					
					1	2	3	1	1	{	0	1	2	2	1	2	{	2	2	0	0	0	0	X							
																		1	2	1	0	X									
																		0	0	1	1	X									
									2		1	1	{	1	0	0	0	0	0	0	X										
												0		0	0	0	X														

X signifies death of the line, while S signifies that the line was discarded while still flourishing. The daily fission rates for the three successive 5-day periods preceding the endomixis beginning December 9th were 1.8, 2.2, and 1.6, respectively.

Other illustrations of this type of change in fission rate were given in our earlier section on hereditary changes in viability.

A second example is illustrated in table 6 by the history of line 036 of clone II. During the three 5-day periods prior to endomixis the mean fission rates of this line were 1.8, 2.2, and 1.6 fissions per day. In a 7-day period, including endomixis, the mean rate was 1.9 fissions. In this period the line was expanded to three lines (1 to 3); subsequently, one of these (3) showed a marked decrease in fission rate and was expanded to five lines (nos. 3 to 7), making a total of seven.

Lines 1 and 2 continued to manifest the usual high fission rate until discarded. Line 2 was discarded 5 days and line 1, 20 days after the beginning of endomixis. At the time it was discarded, line 1 was undergoing another endomixis. During this intermictic period the mean fission rate of line 1 was 2.0 fissions per day. The low fission rate of line 3 first appeared on the fourth day after the beginning of endomixis; this was seven generations after the beginning of endomixis and thus near the end of the process. The change persisted till the death of all five lines (nos. 3 to 7) descended from line 3. These lines died in from 10 to 15 days (mean 12.2 days) after the change. During this time the lines passed through six to fourteen generations (mean 10.6). Their mean fission rate was 0.87 fissions per day, as compared with 2.0 for the unaltered sister lines. During three periods of 5, 4, and 5 days, the mean fission rates of the unaltered lines were 2.0, 2.5, and 2.0, respectively, as compared with 1.1, 0.7, and 0.4 for the sister lines that had become altered during endomixis.

(b) Changes to lower fission rate, lasting through an entire intermictic period and terminating by the death of all the changed lines at the next endomixis. Line 010 affords an example of a change to lower fission rate that lasted until the next endomixis, when all lines died. The mean fission rates of line 010 for four successive 5-day periods beginning November 21st were 2.0, 2.0, 2.0, and 2.0 fissions per day. Fragmentation was observed in this line on December 11th, at which time the line was expanded to two lines (a and b). After the endomictic process had been completed, between December 16th and January 7th, the line 010a was expanded to eight lines and line 010b to seventy-one lines. Comparison between these two groups shows great and consistent differences in mean fission rate. The mean rates for the two groups were determined for five 5-day periods following endomixis (using varying small numbers of lines for each mean, in consequence of the death of some of the lines) as follows:

<i>Period</i>	1	2	3	4	5
Lines 010a	2.00	2.40	2.40	2.05	2.08
Lines 010b	0.80	0.80	0.65	0.60	0.51

As appears from these figures, the highest mean in the course of the five 5-day periods was, for the 010b group, 0.8 fissions per day, while for the 010a group the lowest mean was 2.00 fissions per day. Thus the 010a group reproduced at least two and one-half times as rapidly as the 010b group. In one period (January 4th to 8th) the rate of 010a is four times as great as that of 010b. The difference between these two lines of descent appeared first on December 15th, 4 days and eight generations after endomixis had first been observed. Comparison could be carried no further than January 8th, because within the next 5-day period, all remaining lines of 010b had died. The lines of 010a, on the other hand, continued to live and reproduce as usual; the mean fission rate of the seven lines of 010a for the next three 5-day periods (beginning with January 9th to 13th) were 2.23, 2.46, and 2.10 fissions per day. Deaths began to occur among the lines of 010b on December 22nd, 7 days and four generations after the change in fission rate. These continued until all lines were extinguished by January 14th. Most of the deaths (43 of the 71) occurred, however, after the next endomixis began (January 3rd). After the beginning of this second endomixis, no line lived more than 11 days or passed through more than seven generations. Clearly, therefore, no line of 010b lived beyond this endomixis. Before their death, however, as many as twenty-seven generations had passed at the new fission rate. It should be mentioned that the other line of descent, 010a, passed successfully through the endomixis of January 3rd to 10th, which killed off its sister lines descended from 010b; further, it went through another endomixis on January 20th to 23rd before being discarded on January 25th. The difference between these two lines of descent (010a and 010b), both descended from a single individual (010) undergoing endomixis; the origin of this difference at endomixis; and its persistence until the following endomixis, when it was terminated by the death of all lines, are thus clearly demonstrated. Other examples which show the same type of phenomena could also be given, but they have nothing to add beyond what is illustrated by the above data on the descendants of line 010.

In a number of cases the fission rate of the line appeared to be reduced in small degree after endomixis, to persist thus at a lowered rate through the intermictic period, and to be restored at the next endomixis. To demonstrate such cases requires, however, on account of the only slight lowering of the fission rate, statistics for larger numbers of lines than I have at present data for, so that such cases are reserved for further study.

TABLE 7
Comparison of fission rates of lines 09a and 036a of clone II

(a) PRIOR TO ENDOMIXIS OF DECEMBER 11, 12, 1931						
LINE		Nov. 20-24	Nov. 25-29	Nov. 30- Dec. 4	Dec. 5-9	Mean of means Nov. 20- Dec. 4
09a	Mean fission rate	1.8	2.0	1.8	2.0	1.90
	Number of lines	1	1	1	1	1
036a	Mean fission rate	2.0	2.0	2.0	1.4	1.85
	Number of lines	1	1	1	1	1

(b) AFTER ENDOMIXIS OF DECEMBER 11, 12, 1931											
LINE		Dec. 14-18	Dec. 19-23	Dec. 24-28	Dec. 29-Jan. 2	Jan. 3-7	Jan. 8-12	Jan. 13-17	Jan. 18-22	Jan. 23-26	Mean of means Dec. 14- Jan. 26
09a	Mean fission rate	1.0	1.0	0.9	0.9	0.96	0.8	0.87	0.7	0.7	0.87
	Number of lines	1	8	47	44	40	36	34	32	16	
036a	Mean fission rate	2.4	2.0	2.4	1.6	2.2	2.0	2.1	2.2	2.0	2.10
	Number of lines	1	1	1	2	2	1	2	2	2	

(c) Changes to lower fission rate, lasting through at least two endomictic periods. A marked example of the lowering of the fission rate by endomixis, this lowered rate persisting through more than two later intermictic and endomictic periods, is found in the history of the line 09a of clone II. This line showed many other remarkable changes following endomixis; these are to be considered in later sections. Here we deal only with the change in fission rate. To bring out clearly what has occurred, the history of this line will be compared with that of its sister line 036a (table 1). The essential facts for the two lines are presented in table 7.

Endomixis occurred in these two lines beginning December 11th and 12th. Prior to this endomixis the two lines had practically the same fission rates, as shown by the records for four preceding 5-day periods in table 7, a. The mean daily rates for the 20 days were, for line 09a, 1.90; for line 036a, 1.85.

After the endomixis of December 12th, on the other hand, the fission rates of the two lines were very diverse, as shown in table 7, b. The fission rate of the line 09a fell at endomixis to about one per day, or a little less, while that for 036a remained at about two per day. The lowered rate of line 09a continued for the nine 5-day periods shown in table 7, b, in no case rising above one fission per day. In the course of this 45-day period, the number of lines of 09a was greatly increased, by saving the products of fission, so that the mean fission rate is based, in the period December 24th to 28th, on the data from forty-seven simultaneous lines of the group 09a (the number of lines in the group is given for each period in table 7). In every one of the nine periods the fission rate in 09a is less than half the rate in the lines of 036a; and for the entire 45 days the mean fission rate for 036a is 2.4 times that for 09a.

During the course of the observations, endomixis occurred rather irregularly among the lines of the group 09a. In every 5-day period between January 3rd and January 26th, some of its lines were found to be in endomixis. In some of the individual lines, endomixis occurred in the period January 3rd to 7th, and again in the period January 23rd to 26th. The endomictic period at which the low fission rate was established began December 11th to 12th, as we have seen. Thus the lowered fission rate persisted through two intermictic periods, and through the intervening endomictic period of January 3rd to 7th.

This clone, 09a, was not carried further in isolation cultures. But, as will be set forth later, it was continued in mass cultures, and is still in existence (July 1, 1933), more than a year and 7 months after its origin. During this time it has

retained its peculiar characteristics. Conjugation has never successfully occurred in this stock (see later), so that there is reason to believe that the lines now existing are the vegetative descendants of those produced at the endomixis of December 12, 1931. In any case, the stock has retained its low fission rate. At intervals the fission rate has been determined by carrying a number of lines (in some cases up to twenty-four) for several days in isolation cultures. Immediately after the endomixis at which this stock took origin (December 11 to 12, 1931), it showed a fission rate of 1.00 per day. In 1932, the following daily fission rates were determined for this stock, 09a, at the dates here mentioned (the numbers in parenthesis are the number of parallel lines on which the mean rate is based):

June 26 to 30 (12), 0.85	July 28th to August 1 (7), 1.00
August 10 to 14 (21), 0.91	August 24 to 31 (16), 0.62
October 4 to 9 (4), 1.05	

In July, 1933, isolation cultures were again made of this stock. Nine lines cultivated for 5 days gave a mean fission rate of 0.33 daily. At this time the stock lived well in mass cultures, but most of the lines died out without fission when placed in isolation cultures. The rate just given is based on nine lines that lived and multiplied during the 5 days.

During all these periods, many unmodified stocks were in existence, and these multiplied, under these same conditions, at the rate of two or more fissions daily.

The contrast between the sister lines 09a and 036a, shown in their histories (table 7, etc.), is very great. In the unmodified line 036a the fission rate continued practically uniform from the beginning of observation on November 20th to the end, on January 26th; three intervening endomictic periods did not appreciably affect its fission rate. In the stock 09a, on the other hand, which at the beginning showed the same rate as 036a, the fission rate was reduced at the first endomixis to half the former rate. This lowered rate persisted through the next two endomictic periods, and in mass cultures, up to the present time, more than 18 months later.

This stock 09a changed at the endomixis of December 11 to 12, 1931, in other ways also, notably in size and form; these matters are dealt with in a later section.

A number of other cases similar in character to that just described in 09a were observed, though in some of these the relation of the changes to a particular endomictic period was not so fully determined. In all this work, of course, the observer does not know what the further course of events will be, so that, in the great number of lines to be attended to, details that later turn out to be important are at times overlooked.

TABLE 8

Comparison of fission rates of the stock of 046 with those of the stock 017

		FIRST INTER- MICTIO PERIOD OCTOBER 18-27	SECOND INTER- MICTIO PERIOD NOVEMBER 2-21	THIRD INTER- MICTIO PERIOD NOV. 27-DEC. 8	DEC. 17-21	DEC. 22-26	DEC. 27-31	JAN. 1-5	JAN. 6-10	JAN. 11-15	JAN. 16-20	JAN. 21-25
Lines descended from 017	No.	1	1	1	1	1	4	4	6	2	2	1
	Mean	1.8	1.7	1.9	2.2	2.0	1.6	1.8	2.0	1.8	2.1	2.2
Lines descended from 046	No.	1	1	1	1	1	1	4	4	6	6	3
	Mean	1.7	1.6	2.1	2.6	2.2	2.2	2.1	2.3	2.2	2.0	2.2

(d) Changes to a higher fission rate. In some cases endomixis results in increasing the fission rate in certain lines. Such increase is less common than a decrease due to endomixis, and is not as a rule so great in amount.

An example of such increase is shown in table 8, in the stock 046. To bring out the change, this line may be compared with the stock 017, in which such an increase did not occur at endomixis.

The stocks 046 and 017 were under observation from October 18, 1931, to January 25, 1932, this period covering several endomictic cycles. During the first three intermictic periods the mean daily fission rates were practically identical in the two lines, as shown in table 8. Then came in both lines the endomixis of December 9th to 16th. From this time on, through six 5-day periods (to January 15th) the fission rate

of 046 remains steadily somewhat above that of 017, the latter remaining at nearly the same level as before. The mean daily rate for the six 5-day periods for 017 is 1.90 fission, while for 046 it is 2.27, so that 046 multiplies 1.2 times as rapidly as does 017. This higher rate of stock 046 continued through one endomixis (at December 27th to January 5th in 017 and at January 1st to 5th in 046), and through two inter-mictic periods. Endomixis again occurred in both stocks January 16th to 20th, and after this endomixis the difference between the two sets of lines had disappeared.

In a number of other cases an increased fission rate was observed in certain stocks after endomixis. In many such cases the increase is so slight that there would be necessary, to fully demonstrate it, more extensive statistical data than I have at present available.

(e) Comparison of the frequency of origin of lasting changes in fission rate in the intermictic period with the frequency of origin of such changes during endomixis. Comparisons of lines similar before endomixis and different after it, given in the preceding sections, have to a great extent answered the requirements of controls. Such comparisons demonstrate clearly the altered fission rate appearing in a certain line during endomixis and not appearing in a related line living at the same time. A further control consists in a comparison of the percentage of altered lines arising during the intermictic period with the percentage arising immediately after endomixis. In order to make this comparison, the fission rates of all lines of an intermictic period were examined in successive 5-day periods. 'Altered lines' were defined as those in which the fission rate differed by 0.3 fission per day or more from the modal value for the sister lines of the same period, and in which this change persisted. Such changes of at least 0.3 fission per day were classified, according to the length of time they persisted, into: a) changes lasting 10 days; b) changes lasting through the succeeding intermictic period, or still longer.

Changes lasting 10 days. The data on clones I and II were examined from this point of view. Of 124 lines studied, nine changes lasting 10 days were found to have occurred during the intermictic period, and fourteen during the process of endomixis.

Changes lasting through one or more intermictic periods. No change of this type was found to have occurred during the intermictic period in the 124 lines studied. On the other hand, numerous examples of such changes arising during endomixis have been given in the preceding sections.

Thus during the intermictic period no lasting or truly heritable changes arose, while the considerable number that did arise occurred during endomixis.

3. Origin at endomixis of a biotype (09a) differing in size, structure, and physiology

As described above (p. 392) at the endomixis of December 11 to 12, 1931, the line 09a developed a much lowered fission rate (table 7, etc.). At the same time it developed other marked peculiarities, morphological and physiological, so that there resulted a biotype that was very diverse from those derived from its sister lines. Among these peculiarities were a distinctly reduced size and certain structural changes; also certain peculiarities as to conjugation. An account of these follows:

A. Size. Full-grown individuals of our general stock of *Paramecium aurelia* (all derived in 1930 from a single individual) measured, as a rule, about $177\ \mu$ in length by $80\ \mu$ in breadth. Such was the stock 09a before the endomixis of December 11 to 12, 1931. But after this endomixis the full-grown individuals of 09a averaged about 125 by $64\ \mu$, so that there had been a reduction in length of about 30 per cent, and in width of about 20 per cent. To the eye the difference is very striking, as shown in figure 1, which gives, to the same magnification, outlines of four typical full-grown individuals of 09a, in comparison with a full-grown individual of one of the unchanged stocks, 039h.

Measurements of typical full-grown individuals of the stock 09a, in comparison with full-grown individuals of the typical unchanged stocks 036a, 039b, and 046, were made at intervals for a number of days during January, 1931. These measurements are shown in table 9. In making these measurements, in order to insure that the animals were full grown, individuals were isolated 6 hours before they were killed and measured, so that none of them have recently divided.

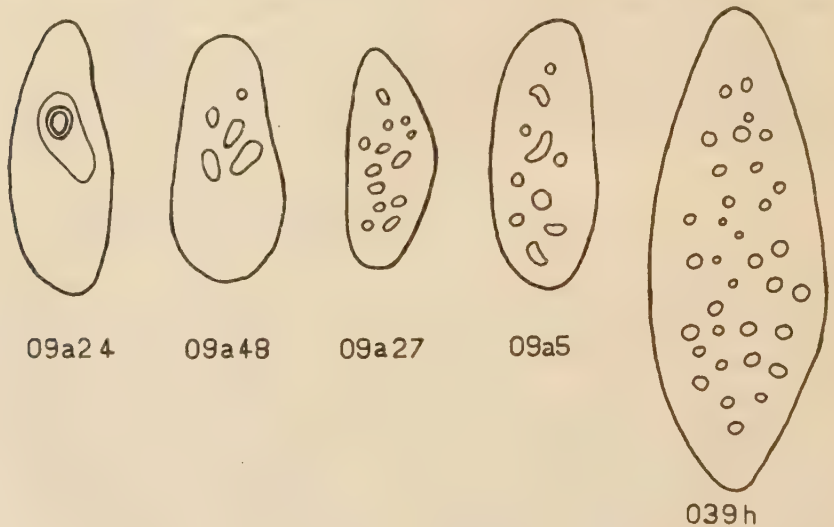


Fig. 1 Outlines, to the same magnification, of four typical individuals of the altered small stock 09a, and of a single typical individual of the unaltered 039h. In the individual 09a24 is shown the intramacronuclear body described in the text. In the individuals 09a48, 09a27, and 09a5 are shown the few fragments of the macronucleus produced at later endomictic periods in the stock 09a, in comparison with the large number produced in the unaltered stock 039h.

As table 9 shows, the length of full-grown individuals of 09a ranged from 53 to 73 units (106 to 146 μ), while in the unchanged stocks the length ranged from 70 to 100 units (140 to 200 μ). The breadth in 09a ranges from 40 to 80 μ ; in the other stocks from 64 to 92 μ .

The greatly decreased size of the members of the stock 09a persisted indefinitely. The decreased length was strongly marked in April, 1933, somewhat more than a year and 3

months after the origin of this greatly changed clone at the endomixis of December 12, 1931. This is demonstrated by measurements of length made early in April, 1933, and shown in table 10. This table gives the lengths of 200 adult individuals of the modified stock 09a, in comparison with the measurements of 200 adults of the unmodified stock H.

With relation to table 10, the following facts are pertinent. During the period from December 12, 1931, to April 1, 1933, the stocks of 09a were kept on isolation slides, or in small

TABLE 9

Lengths and widths of full-grown individuals of three different lines of the small stock 09a (namely, 09a62, 09a64, and 09a68), on successive days in January, 1932, compared with the measurements of full-grown individuals of typical unchanged stocks (036a, 036ab, 039b, and 046) at the same times. The unit of measurement is 2μ , so that each measurement is to be doubled in order to give lengths in microns.

DIVERSE LINES OF CLONE 09a				TYPICAL UNCHANGED CLONES			
DATES	09a62	09a64	09a68	036a	036ab	039b	046
Jan. 15	65×37	63×30		81×46	93×45	93×45	99×44
16	66×35			85×39	95×41	100×45	82×35
17	60×33			80×35	90×37	95×47	88×38
18	65×32			91×40			
19	73×35			100×40	90×43		75×36
20		66×30	70×40		96×40	87×37	82×32
21					95×40		70×35
22	60×40		60×32				
23		60×27	50×27	80×43	91×40		
24	65×30		53×20	90×40	87×40		

mass cultures. So far as could be determined, fertile conjugation did not occur in the stock 09a. During this same period (December 12, 1931, to April 1, 1933) other clones of the same original stock, but from the part not changed in size at the endomixis of December 12, 1931, were cultivated under similar conditions. On March 25, 1933, a single individual from a mass culture of the unchanged stock H (table 1) was isolated, and from it was obtained by fission a large number of individuals constituting a clone. An equal number of individuals of this clone H and of the modified derived clone 09a were used to start two small mass cultures, kept side by side

in the same culture fluid and under the same conditions. After they had been in progress for a day, part of the fluid was interchanged between the two cultures, so that no diversities of bacterial content could occur. After some days of multiplication, more than 100 single individuals were taken from each and placed on isolation slides. They were left on the isolation slides for 6 hours in order to insure that the individuals measured should be adults. At the end of this period,

TABLE 10

Comparative lengths under the same conditions, in April, 1933, of adults of the modified stock of 09a, and of the original unmodified stock H, from which 09a took origin at the endomixis of December 12, 1931. Two independent comparisons are given, as group I and group II, each including 100 adult individuals of each of the two stocks. The unit of measurement is 2μ , so that to obtain the dimensions in microns the measurements are to be multiplied by 2. For convenience of presentation in the table, two units are grouped together. Thus under the dimension 54 are given the number of individuals that measured 53 or 54 units in length (105 to 108μ).

Group I lengths																	Total	Mean
09a Units	52	54	56	58	60	62	64	66	68	70	72	74	76	78	80			
No.	2	4	2	4	4	9	14	11	9	16	11	5	5	3	1		100	66.22
H Units	72	74	76	78	80	82	84	86	88	90	92	94	96	98	100	102	Total	Mean
No.	4	5	5	12	12	5	7	17	6	13	6	4	2	0	1	1	100	83.64

Group II lengths														Total	Mean
09a Units	64	66	68	70	72	74	76								
No.	16	15	15	22	10	12	10							100	68.84
H Units	76	78	80	82	84	86	88	90	92	94	96			Total	Mean
No.	2	2	4	8	14	17	25	14	10	3	1			100	86.37

the individuals that had not divided within the 6 hours were fixed with osmic acid, and 100 members of each group were measured. These constitute the group I of table 10, comprising 100 representatives of the modified stock 09a and 100 representatives of the unchanged group H. To insure against error, another set of each (09a and H) were treated in the same manner, but entirely independently of group I, and 100 members of the modified set 09a and of the unmodified set H were measured. These constitute group II of table 10. We

have therefore, on April 1st, two independent comparisons of length between the modified group 09a, which arose at the endomixis of December 12, 1931, and the unmodified group H from which the group 09a arose. It is these comparative measurements that are given in table 10.

As shown in table 10, adult individuals of the modified stock 09a show, in April, 1933, very nearly the same small size shown more than a year before (table 9). At the earlier period the length ranged from 106 to 146 μ , with a mean of 125.1 μ . At the later period the range is from 104 to 160 μ , with a mean of 135 μ . In the unmodified stocks the mean length at the earlier period is 177.2; at the later period 170 μ . The modified stock 09a is still 20 per cent below the typical stocks in length. The small size that took origin at endomixis has persisted for more than 15 months.

B. Macronucleus. In the early periods of the history of the stock 09a there was a marked peculiarity in the structure of the macronucleus. About 75 per cent of the individuals examined showed a distinctly differentiated spherical body, varying from 5 to 10 μ in diameter, embedded in the macronucleus, usually near the center of that body (see the upper left hand individual in fig. 1). This body is clearly perceived in individuals killed and stained for determining whether endomixis is occurring. Its staining reaction is not noticeably diverse from that of the rest of the macronucleus. But it is sharply demarcated from the rest of the macronuclear material. In some cases two such bodies were found in one macronucleus.

This intramacronuclear body was first observed after the endomixis of December 12, 1931. It was then observed in many of the lines of 09a during January, March, April, and May of 1932.

The stock 09a also showed, after the endomixis of December 12, 1931, a marked peculiarity in the way fragmentation of the macronucleus occurs at endomixis. In the typical stocks fragmentation of the macronucleus during endomixis normally results in the formation of from twenty-five to thirty

bodies of approximately spherical form and of fairly uniform size (fig. 1, line 039h). The number is sometimes greater. Fragmentation in 09a is characterized by a reduced number of bodies, the number being, as a rule, 10 or less. The fragments in this stock often show unusual forms and sizes. In figure 1 are shown the numbers, size, and form of the fragments in four lines of 09a, in comparison with the usual condition in the clone 039h.

C. Physiological peculiarities. As has been described in detail, this stock 09a differed from others in having a much lowered fission rate. It showed likewise other marked physiological peculiarities, as follows.

At endomixis many of the individuals of 09a became unusually pale and some assumed a distorted shape. At one side the body of such individuals was drawn out to form a marked lateral spine, similar to that which is observed after conjugation in some clones. Individuals with such lateral spines died without further multiplication.

Conjugation rarely occurred in members of this stock, and in all cases in which it did occur, the ex-conjugants died without multiplication. In the period April 6th to April 26th, 115 pairs were obtained from 09a, from which 117 ex-conjugants were obtained for cultivation. But all these died without multiplication, though cultivated under conditions in which ex-conjugants from other stocks multiplied vigorously. In June, 1932, sixteen additional pairs were obtained from 09a, but as before the thirty-two ex-conjugants died without multiplication.

After this time (June, 1932) no further conjugations were observed in the cultures of 09a, although they were under daily observation from June to September 15, 1932, and again from May 15 to June 15, 1933. Cultures of related but unchanged stocks showed in this period frequent conjugations. All attempts to induce conjugation in cultures of 09a have failed, from June, 1932, to July, 1933. The stock has apparently lost its power to conjugate.

D. Summary on the new stock 09a. Thus at the endomixis of December 11 to 12, 1931, there originated from a part of one of the clones of this stock of *Paramecium aurelia* a modified stock, showing marked changes in the inherited characteristics. This stock took origin in a single individual that underwent endomixis on the date mentioned. It showed a greatly decreased fission rate, a much smaller size, certain cytological peculiarities of the macronucleus, and a loss of the power to undergo fertile conjugations. This greatly changed stock is still in existence (July, 1933), a year and 6 months after its origin.

RESULTS AND THEIR SIGNIFICANCE

The investigations recorded in the foregoing pages have demonstrated in this stock of *Paramecium aurelia* the occurrence with unexpected frequency of heritable variations at the time of endomixis. Some of the variations last for but a few generations; some result in the death of the lines. Others last through one or more endomictic periods. In some cases they were shown to persist for more than a year. Some of the changes occurring at endomixis affect most or all of the characteristics of the animals: size, structure, vigor, rate of multiplication, power to conjugate. In such cases a biotype is produced that differs greatly from the biotype that gave origin to it.

Are these phenomena of general occurrence in ciliates that undergo endomixis? Or do they result from some peculiarity of the stock with which this study deals? This stock was all derived from a single individual; it has lived under laboratory conditions for 3 years, and it has been much inbred. Whether for these or other causes this stock gives results that are not typical can be determined only by equally careful examination of other stocks and species. But the fact that in some stocks hereditary variations are not infrequent at endomixis is established.

In what way are hereditary variations produced at endomixis? In their paper recording the discovery of endomixis

in *Paramecium aurelia*, Woodruff and Erdmann ('14) suggested that in the nuclear reorganization that occurs at endomixis hereditary variations might arise through 'molecular rearrangements.' This rather undefined possibility has been further discussed in later papers by Erdmann ('20, '22). Erdmann has also suggested that 'recombinations' of the genetic material may occur at endomixis.

New combinations of the genetic material might arise at endomixis in a very simple way, if the parental line were heterozygotic for one or more genes, in case reduction occurred at one of the two micronuclear divisions in endomixis. If the parental line bears the genes *Aa*, some of the lines after the reduction at endomixis would carry only the gene *A*, others only the gene *a*, so that diverse hereditary characters would appear in the different lines.

But after endomixis with reduction had occurred, the clones produced would be haploid with respect to the genes affected by the reduction division. If reduction occurred in the way typical for Metazoa, affecting all the genes, the stocks after one endomixis would be completely haploid. Reduction could not occur again at later endomictic periods. The result would be a long series of haploid vegetative generations. When conjugation occurred it would be without reduction, by the union of two haploid individuals. There is no indication that such phenomena occur in the Ciliata, so that there is no ground for supposing that reduction typically occurs in connection with endomixis.

Thus hereditary diversities arising at endomixis are presumably not the result of typical reduction. They may arise through the 'molecular rearrangements' of Woodruff and Erdmann. Or they may arise through occasional irregularities in the nuclear phenomena. The nuclei go through an elaborate set of processes at endomixis; it would not be surprising if at times this takes an atypical course. This might result in such phenomena as are known in Metazoa under the names 'deficiency,' 'translocation,' 'non-disjunction.' A division that is typically equatorial might at times

become reductional for one or more genes. The hereditary variations that arise at endomixis might be consequences of nuclear irregularities of many different types.

Most of the hereditary variations resulting from endomixis that are described in the foregoing yield biotypes that are less vigorous and that reproduce less effectively than the parental biotypes. Some of them result in distinctly 'abnormal' lines; or result in death within a few generations. In but a few cases were lines produced that exceeded the parental lines in rate of reproduction.

Under usual conditions almost all the aberrant types so produced would soon disappear, leaving only the unchanged lines. Thus the stock as a whole would in the long run be little changed by the variations occurring at endomixis. Only in the rare cases where a more vigorous and rapidly multiplying variant arises would the stock be permanently changed.

The possible significance of the variations occurring at endomixis for the production of new types and for adaptation to new environments has been fully discussed by Erdmann ('20, '22), so that the matter need not be taken up in detail here. The facts brought out in the present paper fit remarkably well into the conception set forth by Erdmann.

SUMMARY

The production of inherited variations at endomixis was studied in an inbred stock of *Paramecium aurelia*, derived from a single individual in 1929, and cultivated for 3 years in the Zoölogical Laboratory of The Johns Hopkins University.

I. Viability

1. In certain clones many non-viable lines are produced at endomixis. In such clones mortality during the ten generations following endomixis is many times greater than at other periods.

2. Some lines are differentiated at endomixis into viable and non-viable descendant lines.

3. Different clones entering endomixis show great diversities in the number of non-viable lines produced.

4. Some clones go through endomixis with undiminished vigor and without deaths in any of their branches.

II. Fission rate

5. The variability in fission rate in some cases increases greatly as the lines constituting a clone passes through successive endomictic periods. Lines of characteristic higher and lower rates are produced at these periods.

6. Lines that are practically identical in fission rate before endomixis at times emerge from endomixis with diverse fission rates.

7. Most of the changes at endomixis are to a lower fission rate. Some of the changed lines continue for but a short time, dying out within a few generations. Others persist throughout the entire succeeding intermictic period, but all die during the next endomixis. In others, lowered fission rate persists through several succeeding endomictic periods; it apparently continues indefinitely.

8. In rare cases there is at endomixis a change to a higher fission rate.

III. Origin of new biotypes

9. In one case that is described there arose at endomixis a biotype (09a) differing from the original stock in many morphological and physical features. It had a much smaller size, an altered macronuclear structure, a lowered fission rate and lowered viability, and had lost the power to undergo fertile conjugation. This altered biotype is still in existence more than 18 months from the time of its origin.

The general result is to demonstrate an unexpected frequency of heritable variations at endomixis.

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VITA

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